

# Monkeypox Virus

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## INTRODUCTION

At this time in the history of smallpox a major effort is being made to eradicate the disease from people in geographic areas of high prevalence (2). Variola is considered a disease transmissible from person-to-person; no other reservoir of the virus responsible for smallpox has been identified. The recent discovery of a closely related pox disease in monkeys (70), called "monkeypox" has provoked inquiries regarding clinical and epidemiological relationships of these two diseases. In its clinical aspects monkeypox is analogous to smallpox, or to generalized vaccinia developing in human beings.

In relation to the epizootiology of monkeypox several important questions arise regarding its origin. Did it originate accidentally in captive

monkeys from variola virus harbored by a human associate? Is it a variant of variola virus adapted long ago to simians, and now enzootic in certain species? Possibly the same questions could be asked with respect to vaccinia virus. Finally, address is made to the query whether or not monkeypox might be caused by another previously undefined member of the variola-vaccinia complex. Additionally, epidemiological implications must per force be brought into focus, for natural infection from monkeypox virus (MPV) has been reported in human beings (3, 4).

Fourteen years have elapsed since the recognition of MPV. From a nosographic point of view, this disease in a nonhuman primate has proved to be a very satisfactory model for a study of the variable characteristics of infection and immunity of a poxvirus infection. The

purpose of this review is to bring to the fore available information concerning (i) MPV and (ii) the evolution of the disease in simian species, and (iii) to reexamine the epidemiological potential with respect to an extrahuman reservoir of another poxvirus capable of causing human illness.

## HISTORICAL NOTE

### Recognition of Monkeypox in Nonhuman Primates

Smallpox has been a disease of major importance to man for many centuries (25). Naturally occurring epidemics of pox diseases among nonhuman primates have occasionally been reported. In their survey in 1968, Arita and Henderson found seven recorded episodes of pox diseases in nonhuman primates; one of these was confirmed by virus isolation (6).

In 1958, von Magnus et al. (7) observed two outbreaks of a nonfatal poxlike disease in two shipments of cynomolgus (*Macaca cynomolgus*) monkeys arriving in Copenhagen after shipment from Singapore. A poxlike skin eruption developed among the animals between 51 and 62 days after arrival in Copenhagen. Approximately 20 to 30% of the animals developed clinical illness. The epizootics in Copenhagen suggested slow recruitment of susceptible monkeys in a cycle of inapparent infections with subsequent intensification and the emergent clinical expression of monkeypox. Whether the risk of disease was dependent upon intensified replication of MPV in vivo or enhanced invasiveness, or both, is unknown. We assume that the primary source of MPV came from a companion monkey; whether MPV came from a recent (nasopharyngeal colonization) or remote (latent carrier of virus in tissues) infection is also unknown. The latter kind of origin is possible since a virus analogous to MPV has been recovered from the kidneys of apparently healthy monkeys (quoted in 5, 6, 31).

Viruses isolated from the dermal lesions of affected monkeys produced pock lesions on the chorioallantoic membrane (CAM) of developing chicken embryos, cytopathic effects (CPE) in mammalian cell cultures, encephalitis in mice, and dermal lesions, as well as keratitis, in rabbits. Morphologically, the prototype virus had the rectangular shape and size typical of other known poxviruses (200 by 250 nm); antigenically, it was closely related to the vaccinia-variola subgroup of poxviruses (Table 1). Since it appeared to differ from variola and other known poxviruses, it was named mon-

keypox virus and given recognition as a specific member of the group.

In 1959, an outbreak of monkeypox occurred in the animal quarters of Merck, Sharp, and Dohme in Philadelphia (63, 64, 67). Within a colony of 2,000 monkeys (56% *Macaca mulatta*, 41% *Macaca philippinensis*, and 3% *Cercopithecus aethiops* var. *subaeus*) at least 10% of companion monkeys were considered to have been infected. Less than 0.5% of those affected died. The affected monkeys were predominantly *Macaca philippinensis*, although *Ma-*

TABLE 1. Classification of the poxvirus group<sup>a</sup>

Subgroup	Infections in nature	
	Man	Animals
I. Variola-vaccinia viruses		
Variola major (smallpox) .....	+	Monkeys (?)
Variola minor (alastrim) .....	+	
Vaccinia .....	+	Calves, sheep
Cowpox .....	+	Cattle
Monkeypox .....	+	Monkeys
Ectromelia (mousepox) ..	+	Mice
Rabbitpox .....	-	Rabbits
Buffalopox .....	-	Buffalo
II. Orf-like viruses		
Bovine papular stomatitis .....	-	Cattle
Contagious pustular dermatitis (orf) .....	+	Sheep
Milker's nodules .....	+	Cattle
III. Avian poxviruses		
Canarypox .....	-	Canary
Fowlpox .....	-	Chickens
Pigeonpox .....	-	Pigeons
Turkeypox .....	-	Turkeys
IV. Myxoma-fibroma viruses		
Rabbit myxoma .....	-	Rabbits
Rabbit fibroma .....	-	Rabbits
Squirrel fibroma .....	-	Squirrels
Hare fibroma .....	-	Hares
V. Unclassified poxviruses		
Molluscom contagiosum .....	+	
Yaba tumor virus .....	-	Monkeys
Goatpox .....	-	Goats
Sheeppox .....	-	Sheep
Swinepox .....	-	Swine
Entomopox (insect viruses)		
Horsepox .....	+	Horses
Camelpox .....	+	Camels
Tanapox .....	+	

<sup>a</sup> See references: 9, 26, 54, 65.

*caca mulatta* also developed clinical evidence of disease.

During 1962, monkeypox was recognized in the primate colony of the Walter Reed Army Institute of Research, Washington, D.C. (49). The disease was observed initially in a cynomolgus monkey which had been given total body irradiation. Subsequently, similar lesions developed in two other cynomolgus monkeys; of the two, one had been irradiated; the other, an apparently healthy animal, was untreated. Both irradiated monkeys died of the disease, whereas the healthy animal survived. Monkeys in the colony were studied serologically in order to determine rates of past infection. Among 27 cynomolgus monkeys exposed to MPV, 25 (93%) had specific antibodies, whereas, in another group of 45 cynomolgus wherein exposure had been unrecognized, only 5 (11%) had specific antibody. Fifty-two of 67 rhesus, and 6 of 14 African green monkeys were also seropositive. While it is likely that clinically inapparent infection occurred widely among exposed monkeys, no pre-enzootic sera were tested; hence rates of seroconversion remain unknown.

In 1964, Peters reported an outbreak of monkeypox in the Zoological Garden, Rotterdam, Netherlands. The affected animals included giant anteaters (*Myrmecophaga tridactyla*), orangutans (*Pongo pygmaeus*), chimpanzees (*Pan troglodytes*), gorillas (*Gorilla gorilla*), guenons (*Cercopithecus* sp.), squirrel monkeys (*Saimiri sciurea*), macaques (*Macaca* sp.), gibbons (*Hylobates lar*), and marmosets (*Hapale jacchus*) (31, 60, 61). Eleven of the 23 affected animals died, including 6 of 9 orangutans, 3 squirrel monkeys, 1 gibbon, and 1 marmoset. MPV was isolated from 1 of the anteaters, 7 orangutans, and 3 monkeys of various species (31). During 1964 and 1965, three silent MPV infections were recognized in colonies of cynomolgus monkeys at Utrecht, Netherlands. These infections were recognized by recovery and identification of a virus obtained from kidney cells cultured from apparently healthy monkeys (31).

In 1967, the World Health Organization (WHO) surveyed 26 major biological institutes in which large numbers of monkeys were used to study outbreaks of monkeypox. Besides the five outbreaks confirmed by virus isolation mentioned above, there were in the U.S.A., between 1965 and 1967, four other instances of poxlike disease compatible with monkeypox; none of the diseases was confirmed virologically (6).

Marennikova et al. (48) subsequently compared the properties of five strains of MPV;

four of the five MPV strains tested were similar in biological properties and could be readily distinguished from both variola and vaccinia viruses. A fifth strain differed from the other four in several characteristics and had properties indistinguishable from variola virus.

#### Human Infection with Monkeypox Virus

Six human infections from MPV were reported in 1970 from the Democratic Republic of the Congo, Liberia, and Sierra Leone (3, 4). All six patients were unvaccinated; each illness was diagnosed as smallpox based on clinical features of infection. The agents recovered from these patients were studied intensively by several reference laboratories and each was identified as MPV. The first case occurred in a 9-month-old infant residing in the Democratic Republic of the Congo. The four affected children in Bouduo, Liberia, ranged from 4 to 9 years of age. Three children who were playmates developed rashes on successive days, thus suggesting common exposure. The fourth child resided 12 miles distant from the others. The sixth case occurred in Sierra Leone; the patient, a 24-year-old male, had removed the stomach and intestines from a "red monkey" 3 to 4 weeks preceding onset of illness. None of the patients died of monkeypox. Moreover, no other member of the households of these patients developed the disease. According to Henderson, another case had been discovered since the last report (41).

Large numbers of monkeys and apes inhabit the affected areas; they are frequently killed and eaten by people, and their skins are used in households. Five of 18 monkeys (species unstated) captured near Bouduo yielded sera containing low titers of hemagglutination inhibition (HI) antibody against MPV. Four of 16 chimpanzees captured in Sierra Leone were also seropositive. In striking contrast, sera obtained from 55 monkeys (species unknown) in Nigeria where human infection is unrecognized were seronegative. Additionally, sera obtained from several thousand African and Asian monkeys have apparently failed to neutralize MPV.

#### CLASSIFICATION

At the present time there is no generally accepted overall classification of the poxviruses. The commonly used classifications are listed in Table 1. The available evidence indicates that MPV belongs to the subgroup of variola-vaccinia viruses. An important feature of this subgroup of viruses is that recovery from

infection with one member confers immunity to another. Because of the lack of apparent differences in physical and chemical properties among members of the variola-vaccinia subgroups, biological properties have frequently been used to distinguish one from the other (25, 29). The major distinguishing features of MPV and other poxviruses are listed in Table 2. On the basis of these data, MPV has properties placing it in an intermediate position between variola and vaccinia viruses. However, studies of MPV in monkeys in the CAM of developing chicken embryos and of its ceiling temperature suggest that MPV is more closely related to variola than to vaccinia virus (11, 18). Failure of pock formation at 40.5 C has been used to differentiate MPV and variola from vaccinia, cowpox, and rabbitpox viruses (11).

In addition to MPV, other poxviruses also produce poxlike diseases in nonhuman primates. These include smallpox (6, 13, 36, 58, 59), Yaba tumor virus (10), Yaba-related virus (15, 22, 38, 53, 57), and molluscum contagiosum (27). Most species of monkeys are not very susceptible to experimental infection with smallpox virus (5, 14, 36, 58). Natural infection is also infrequent (6). Clinical distinction between smallpox and monkeypox, both in monkeys and in man is not possible (3, 75). Some outbreaks of poxlike diseases in monkeys, ascribed to but unverified as smallpox, may relate to infections caused by other viruses, e.g., monkeypox or herpesviruses (24). Natural infection with Yaba monkey tumor virus has been reported only once in Yaba, Nigeria, in 1957 (10). Other encounters with this tumor virus have primarily related to experimental infection in monkeys and accidental or experimental infections in human beings (1, 5, 32, 33, 43; Mira and Sheek, personal communication).

In 1966, three separate outbreaks of infection from a Yaba-like virus occurred at about the

same time in primate colonies located in California, Oregon, and Texas (15, 38, 57). In each instance the affected monkeys had been received from one distributor. Animal caretakers, particularly those handling monkeys, developed cutaneous lesions similar to those encountered in affected animals (22, 53). The cutaneous expression was characterized by the development of a solitary lesion in contrast to the disseminated eruption of monkeypox. Similar solitary lesions have been encountered among human beings during outbreaks of Tanapox (26). Molluscum contagiosum has been recognized in chimpanzees (27).

## MONKEYPOX VIRUS AS AN INFECTIOUS AGENT

### Physical and Chemical Properties

Published information concerning physical and chemical properties of MPV is limited in scope. MPV has the morphological characteristics of other poxviruses. It is brick-shaped with a size of about 200 by 250 nm (55, 63, 70). The virus is resistant to ether and relatively resistant to dessication both in heat and cold. Heat stability tests indicated that 20 min of heating at 40 C caused no significant loss of infectivity, whereas 20 min of heating at 50 or 56 C resulted either in almost complete (92.3%) or complete loss of infectivity (66). Repeated freezing and thawing up to 12 times produced a loss of only 0.2 to 0.6 log<sub>10</sub> of infectious virus. The stability of virus stocks stored at different temperatures was studied over a 15-month period. At the end of 6 months, the infectivity titer of stocks held at 4 C remained unchanged from the original; however, at -20 C there was a 2.2 log<sub>10</sub>, and at -70 C a 1.5 log<sub>10</sub> loss of infectious virus. By the end of 15 months of storage, the loss was 2.5 log<sub>10</sub> at 4 C, 3 log<sub>10</sub> at -70 C, and more than 4 log<sub>10</sub> at -20 C (66). Various chemicals such as

TABLE 2. *Biological properties of variola, vaccinia, and monkeypox viruses* (16)

Properties	Variola	Vaccinia	Monkeypox	References
Lesion on CAM <sup>a</sup>	Small	Large	Small	31, 64, 70
Maximum temperature for growth on CAM	38.5 C	41.0 C	39 C	11
Dermal lesion in monkey	Generalized	Local	Generalized	37, 75
Rabbit skin passage	No	Yes	Yes	31, 49, 64, 70
Lesion in rabbit skin	Not hemorrhagic	Not hemorrhagic	Hemorrhagic	31
Pathogenicity in 3-week-old mice by i.c. route	No	Fatal	Fatal	49, 64, 70
Plaque formation in chicken embryo cell culture	No	Yes	Yes	51, 56

<sup>a</sup> CAM, Chorioallantoic membrane of chicken embryo.

formaldehyde, sodium dodecyl sulfate (SDS), chloroform, methanol, and phenol also inactivate MPV (66, 70).

### Growth Characteristics in Cultured Cells

**Cytopathic effect (CPE).** A broad spectrum of cultured cells is permissive of growth of MPV with an associated cytopathology. These cultured cells include primary and secondary lines of kidney cells derived from rhesus, cynomolgus, and African green monkeys (20, 49, 66, 70); cells from rabbit, bovine (49, 66) and guinea pig kidneys (48); mouse liver cells (66); and some of human origin, i.e., amnionic and human lung fibroblasts (31, 48, 66, 70). CPE has not been observed in our laboratory in Hep-2 cells, certain lines of HeLa cells, and chicken embryo cell cultures (66), although others (48, 70) have reported CPE in these cell cultures. Marennikova et al. (48) reported that a strain of MPV "64-7275" had all the properties of variola virus and was unable to replicate in a continuous line of pig embryonic kidney cells which supported four other strains of MPV.

The CPE of MPV in most cell cultures is characterized by rounding up, granulation, and condensation of cells and then final detachment from the side of the glass, leaving microscopic visible "holes" in the residual cell monolayer (20, 70). Affected cells in monkey kidney and human amnion cell cultures are interconnected by threadlike syncytial elongations, but such cellular bridges are not apparent in HeLa cells (70). The time of appearance of CPE in MPV-infected cell cultures is a function of multiplicity of infection. Depending upon the size of inoculum, CPE of MPV-infected CV-1 cells (a continuous line of African green monkey kidney cells) may be observed as early as 8 h or as late as 10 days or more (20). When the suspension of pustular material from infected monkeys is inoculated into such tissue cultures, the CPE usually develops in 2 to 3 days. Complete destruction occurs after 5 days of incubation (70). In tissue cultures the infectivity titers of most of the passage fluids vary between  $10^{-4}$  and  $10^{-6}$  50% tissue culture infective doses (TCID)<sub>50</sub>/ml (20, 70).

The physical characteristics of CPE produced by MPV in monkey kidney-cultured cells cannot be distinguished from those of variola (31) and vaccinia viruses (20).

**Plaque formation.** MPV regularly forms plaques in a variety of cultured cells (20, 51, 56, 66). Generally, tissue culture cells that give rise to CPE also form discrete plaques (66). When

MPV-infected monolayers of monkey kidney cells grown in petri dishes are overlaid with agar and stained with neutral red, well-defined plaques 2 to 3 mm in diameter can be regularly demonstrated. Thus, the plaque methods can be used for relatively precise quantitative studies of MPV (17, 20, 66).

It has been suggested that MPV may be differentiated from variola virus by the smaller size of the plaques (51) and by an ability to MPV to form plaques in chicken embryo fibroblasts (51, 56). Attempts to induce plaque formation in chicken embryo fibroblasts with both variola and alastrim have failed (56). The inability of MPV to form plaques in chicken embryo fibroblasts has also been reported by others (66).

**Viral multiplication.** Studies on the course of MPV infection in tissue culture cells indicate that the kinetics of intracellular replication—namely synthesis of viral antigens, changes in cell morphology, formation of inclusion bodies, and release of virus from cells—resemble those of vaccinia (23, 30, 68, 69) and variola viruses (34, 62). The minor variations noted between these viruses may be attributable to type of cell culture, multiplicities of infection, and conditions of cell growth, among other factors.

After inoculation of an appropriate quantity of virus into primary rhesus monkey kidney cells or kappa cells (a continuous line of rhesus monkey kidney cells), the majority (75 to 85%) of the virions are attached to cell receptors within 2 h and all (100%) are similarly attached within 4 h (66). The uncoating process begins at 2 h in monkey kidney cells, and synthesis of messenger ribonucleic acid is necessary for the primary uncoating (79). The detailed biochemical events associated with MPV replication are unknown; presumably, they are similar to those of vaccinia virus (44, 78).

A one-step growth curve of MPV in CV-1 cells (Fig. 1) using a multiplicity of infection of 2 plaque-forming units (PFU)/cell revealed a 6-h period of partial eclipse, presumably representing the period of attachment, uncoating, and synthesis of the earliest virions. Thereafter, virus replication occurred at an incremental rate until exhaustion of available substrate by the 14th hour. The pattern of increase of "cell-free virus" followed closely that of cell-associated virus, with a lag of 3 or 4 h between intracellular maturation and release into extracellular milieu. The time required for MPV to reach maximal levels of infectivity varied with multiplicity of infection and type of cell

culture used in tests (20, 66). Release of virus from infected cells into culture fluid is approximately 1% for kappa cells (66) and 10% for CV-1 cells (20).

By using the immunofluorescent method, MPV antigens can be revealed in infected CV-1 cells just before detectable increases of infectious virus (Fig. 1). Cytoplasmic immunofluorescence is the general rule, but, not infrequently, antigens may be present also in the nuclear area or in long cellular bridges of infected cells (20). Inclusion bodies can be demonstrated readily in MPV-infected cell cultures (20, 31, 63). The development of cytoplasmic inclusions in MPV-infected monkey kidney cells coincides with the kinetic aspects of viral replication (Fig. 1). The inclusions appear to contain both deoxyribonucleic acid and other viral antigens as shown by histochemical and immunofluorescent staining (20).

### Growth on the Chorioallantoic Membrane

MPV produces pocks on the CAM of developing chicken embryos which are quite similar to those of variola virus; the pocks are consistently smaller in size than those of vaccinia virus (18, 31, 70). Inoculation on the CAM of dermal pustular material, or blood obtained from monkeys infected with MPV, was followed initially by edematous clouding of the membrane and development of small discrete pocks (18, 70). On serial passage of the CAM-adapted virus, the edematous reaction subsided and small opaque dome-shaped pocks were formed. The titer on CAM of the original pustular materials may reach levels as high as  $10^8$  PFU (70). In general, pocks on the CAM are nonhemorrhagic (18, 31, 70); however, pocks with hemorrhage in the center have been encountered. Four strains of MPV studied by Marennikova et al. (48) ("Copenhagen" "65-31," "65-32," and "7-61") produced pocks with hemorrhagic centers, whereas one strain ("64-7275") was consistently nonhemorrhagic.

MPV forms pocks on the CAM when incubated between 33 C and 39 C (11, 18). The maximum temperature permissive of pock formation on CAM varies for members of the poxvirus group (11); the ceiling temperature range has been reported as follows: alastrim 37.5 C, variola 38.5 C, monkeypox 39.0 C, ectromelia 39.0 C, cowpox 40.0 C, vaccinia 41.0 C, and rabbitpox 41.0 C. These ceiling temperatures have been used as one of the means of differentiating strains of poxviruses within the vaccinia-variola subgroups.

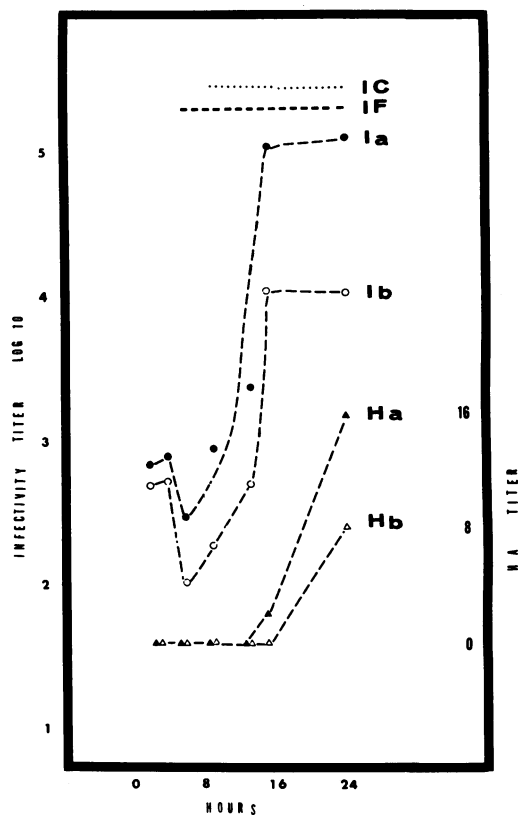


FIG. 1. One-step growth curves of MPV in CV-1 cells (20). The data were derived from multiplicity of infection of 2 PFU/cell. Infectivity titer is expressed in  $\log_{10}$  PFU/0.1 ml (Ia, cell-associated virus; Ib, cell-free virus). Hemagglutinin titer is expressed as reciprocals of dilution of infected culture (Ha, cell-associated fraction; Hb, cell-free fraction); (top) the temporal course of development of cytoplasmic inclusions (IC) and of cellular immunofluorescence (IF). Reproduced by permission (Proc. Soc. Exp. Biol. Med. 139:1206-1212, 1972).

### Hemagglutinin Production

MPV, like some other poxviruses, has an associated hemagglutinin (HA; see references 20, 21, 29, 44, 48, and 70). The (HA) of MPV agglutinates erythrocytes obtained from chickens, but not from mice (70). Among five strains of MPV tested, four strains produced HA titers between 1:64 and 1:128 (48). Another strain produced only a low titer of HA (1:4) similar to that of variola virus. Production of HA is largely dependent upon the type of cell culture employed for infection. During the initial studies of the "Copenhagen strain" by von Magnus et al. (70), no HA was demonstrable; however, in later studies of other cell cultures, HA was

detected (20, 48). The titer of HA is generally higher in extracts from infected CAM than from tissue culture fluids (48, 70). Finding that the amount of HA produced by the same virus strain varies in different tissue systems used for viral growth suggests that, although the viral genome contains information to cause synthesis of HA, the information necessary for its synthesis resides in the genome of the host cell (44).

HA of MPV is present in both "cell-free" and "cell-associated" fractions of MPV-infected cells and increases in titer value during the late phase of virus multiplication (Fig. 1). HA of MPV resembles that of vaccinia virus (46) where HA becomes detectable only after mature virus has accumulated. As with other poxviruses (44), the HA of MPV appears to be a nonessential part of the virion, since dissociation of infectivity and HA activity can be clearly demonstrated by sucrose density centrifugation (Cho, unpublished data). Viral particles separated in the sediment contained little HA and high infectivity, whereas the "top-band" of the supernatant fluid contained abundant HA and little infectivity.

#### Antigenic Composition

MPV has a complex antigenic structure. This virus shares with vaccinia and variola viruses common structural and soluble antigens (31, 70, 77). Studies on antigenic relationships between vaccinia, variola, and monkeypox viruses by HI, complement-fixation (CF; see reference 70), and by diffusion-in-gel precipitin tests (31) have not revealed readily recognizable differences between them (70). Vaccinia virus and MPV are neutralized to the same extent by MPV antisera (40). Cross-reactivity between vaccinia virus and MPV has also been observed by immunofluorescent antibody labeling using either conjugated vaccinia or MPV antibody (Cho et al., unpublished data).

On the other hand, Prier et al. (63) in cross-CF tests of vaccinia and monkeypox sera found that homologous exceeded heterologous titers, suggesting differences in their soluble antigens. von Magnus et al. (70) also reported that when monkeypox and vaccinia viruses were tested against vaccinia antisera by agar precipitin methods, five precipitin bands developed for vaccinia virus, whereas only four such bands developed for MPV. Although these viruses share many common antigens, minor antigenic differences may exist between MPV and vaccinia virus; these differences require further elucidation.

## INFECTIONS IN ANIMAL HOSTS

### Host Range

Studies on the host spectrum of MPV in tissue culture cells reveal that both primary and secondary cell lines derived from many species (i.e., mouse, rabbit, dog, bovine, monkey, and human) support the growth of this virus (66). In this respect, MPV resembles variola (25, 34, 62) and vaccinia (25) viruses studied in tissue culture systems. Although MPV produced CPE in a variety of mammalian cell cultures, the virus is not pathogenic for many of the animal species from which these cells are derived. Similarly, variola virus multiplies in many types of cultured cells, and like MPV has a very limited host range with respect to naturally occurring, or experimental, infections (34, 36).

MPV appears to have a wider host range than variola virus (5). Among primates the susceptible monkeys include cynomolgus (49, 63, 70, 75), rhesus (49, 75), African green (49, 60), marmoset, and squirrel monkeys (60); apes, orangutans, gibbons, gorillas (40, 60), and chimpanzees (52, 60) may also be involved by disease. Susceptible nonprimate animals include the giant anteater (60), rabbit (31, 48, 63, 70), mouse (63, 70), chicken embryo (17, 18, 31, 48), and guinea pig (63). As noted earlier, human infections have been recognized (3, 4, 41).

### Routes of Transmission

Monkeypox has developed in monkeys following intradermal, subcutaneous, intramuscular, and intravenous inoculation of MPV (31, 37, 58). Clinical and subclinical infections regularly develop among uninoculated companion monkeys separately caged among experimentally infected animals (58, 73, 75). The natural route of these latter infections is presumably by the respiratory pathway (37), although autoinoculation or ingestion of viral particles, or both, are possible portals of entry.

### Clinical Manifestations

The descriptive clinical features of monkeypox have been recorded during naturally occurring and experimental infections (49, 60, 63, 70, 73, 75). As a rule, the disease is generalized with the development of a rash of varying severity in different species of primates. In almost all respects it resembles naturally occurring variola in man (25, 65) and experimental variola in monkeys (14, 35, 37, 42, 47, 59, 76).

The incubation period of the disease in experimentally infected animals has varied from 7 to 14 days in cynomolgus and rhesus monkeys, and in baboons (40, 73, 75). In cynomolgus monkeys fever is the first clinical sign, usually of abrupt onset on the 3rd day after virus inoculation (Fig. 2). Shortly thereafter, a generalized lymphadenopathy develops and the mucocutaneous lesions generally appear between the 7th and 14th day. The eruptions are usually seen on the face, trunk, extremities (particularly on palms and soles), tail, and oral mucosae. The lesions on the skin rapidly pass through the stages of papule, vesicle, pustule, and crust. The papular and vesicular stages are brief, lasting only 1 or 2 days. The pustular stage lasts about 2 days; crusting ensues and persists for about a week, or longer if pyoderma intervenes.

Cynomolgus monkeys readily develop clinical disease; they express disease much more

intensely than rhesus monkeys, both in naturally occurring outbreaks (49, 63) and in experimental infections (75). The baboon seems to rank between the rhesus and cynomolgus monkeys with respect to clinical susceptibility (40). Subclinical infections probably occur during epizootics more commonly than is currently recognized, for, as noted above, antibodies may develop in the absence of clinical disease (40, 63, 70, 75). As noted earlier, MPV has been recovered from the kidneys of apparently healthy monkeys (5, 6, 31).

The mortality rate of monkeypox varies from less than 3% (40, 63, 70, 75) to 48% (60) and is dependent upon species susceptibility, the biological pressures applied in experimental infections and in some instances, on interaction and intervention of other microorganisms leading to bacterial septicemia. The mortality rate is greatly increased in animals receiving immunosuppressive treatment either after ir-

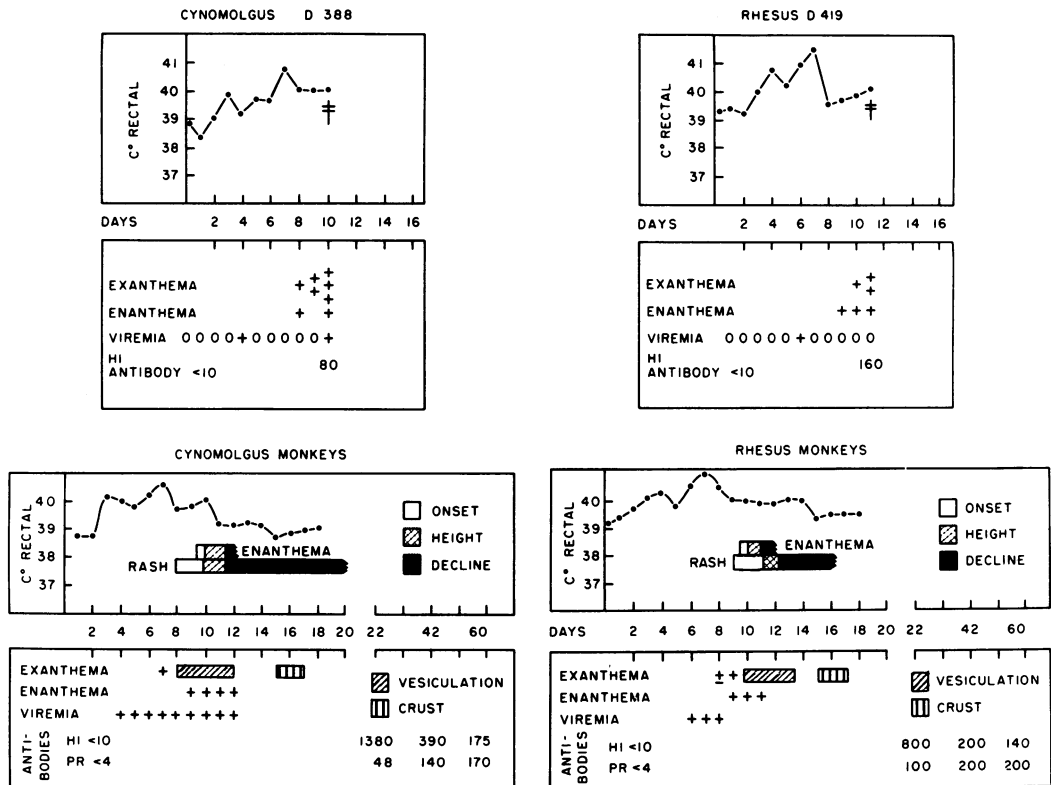


FIG. 2. Clinical and laboratory features of cynomolgus and rhesus monkeys inoculated intramuscularly with MPV (75). Top panels pertain to typical findings for a cynomolgus and rhesus monkey. Bottom panels pertain to the composite picture obtained from both species. The temperature values are expressed as the arithmetic values for at least nine monkeys on the stated days; antibody (geometric mean values) expressed as the reciprocal of the serum dilution end point. Reproduced by permission (Amer. J. Epidemiol. 87:551-566, 1968).



radiation (49) or after the use of antilymphocytic sera (ALS) (72). Experimentally infected animals were given ALS, a principal cause of death related to bacterial infection, intervening at about the 3rd week during post-eruptive phase of monkeypox. Apparently, during this phase bacteria within dermal or intestinal lesions penetrate the blood stream which leads to septicemia and death. In the Rotterdam Zoo (60) an outbreak among monkeys and apes was also complicated by fatal bacterial infections. A variety of bacteria have been recovered including several species of staphylococci, streptococci, enterobacteria, pseudomonas, proteus, and enterococci (60, 71, 72). Not all animals necessarily die of intervening bacterial infection; Heberling et al. (39) observed that germ-free baboons had an accelerated clinical course with leukopenia and died of monkeypox, whereas conventionally reared baboons with leukocytosis survived the infection. In both sets of baboons HI antibody developed by the 10th day after infection.

### Pathogenesis

In monkeys, monkeypox is analogous to variola and generalized vaccinia in man. Provided with the right experimental conditions, we have shown that MPV regularly produces dis-

ease in susceptible cynomolgus monkeys. When inoculated intramuscularly, one TCID<sub>50</sub> is sufficient to produce infection (73).

Pathogenetic studies using cynomolgus monkeys infected intramuscularly (71, 73) indicate that the earliest multiplication of MPV takes place in local cellular components, probably fixed or wandering cells of connective tissue. The sequential events of pathogenesis of monkeypox appear to develop in an orderly way (Fig. 3). MPV is first detected at the local site of infection and is associated with an intense inflammatory response characterized by cell necrosis, phagocytosis, vasculitis, and local replication of MPV. These early cellular reactions appear to be the forerunners of transport of virus to other cellular loci through regional lymphatics and vascular channels (primary viremia). MPV is transported in lymph to regional lymph nodes and very likely in blood to spleen, tonsils, and bone marrow. These organs comprise, among others, secondary sites of virus multiplication, and with further release of virus there is a consistently measurable level of viremia; presumably at this stage, the virus is transported to tertiary target organs (skin, testes, etc.) resulting in clinically recognizable disease. In most respects the pathogenesis of monkeypox generated either experimentally or in sentinel animals follows similar patterns to

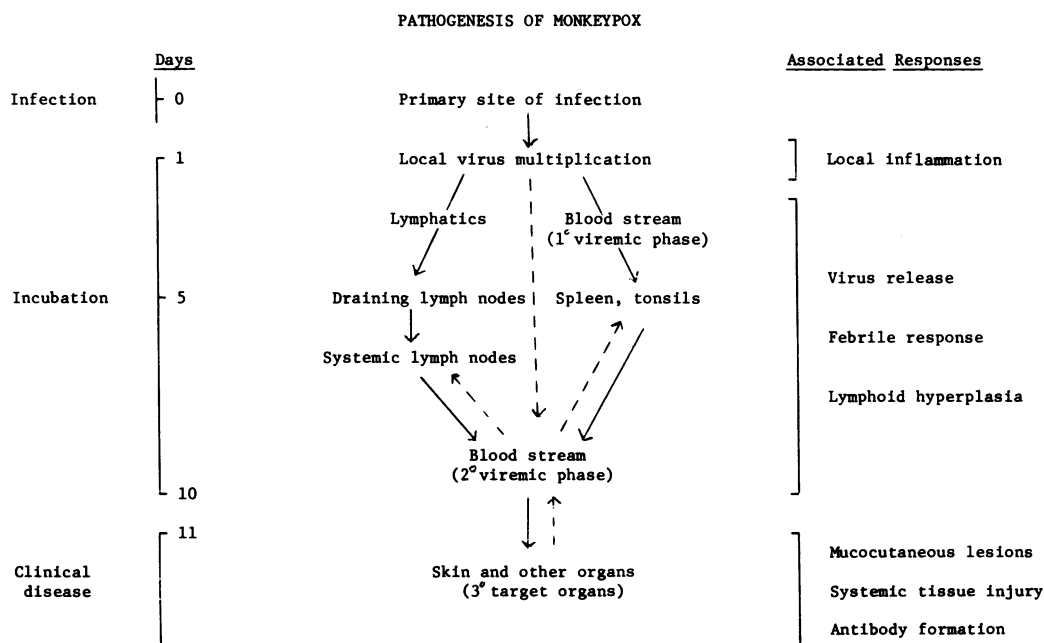


FIG. 3. Model for the pathogenesis of monkeypox (16, 73). Model is based on data derived from MPV infected intramuscularly in cynomolgus monkeys.

those described for ectromelia (28), rabbitpox (12, 19), and variola (35).

The evidence supporting the association of the inflammatory lesions of the skin and other organs with MPV is based on several documented facts; among the first of these is the high concentrations of virus recovered from affected tissues (71, 73). Moreover, foci clearly labeled by fluorescent antibody (indicating the presence of viral antigen) when restained with hematoxylin and eosin, or other stains, reveals not only an inflammatory response, but intracytoplasmic inclusions as well (71, 72). Although a strong stand can be taken in defense of a direct attack by MPV on selected cells, we must point out that antibodies appear at about the same time as detectable cell injury, suggesting (remotely to us) that a part of the inflammatory response may relate to antigen-antibody interactions.

### Viremia

In cynomolgus monkeys viremia is a constant feature of infection (73, 74, 75; Fig. 2 and 4) and appears to be responsible for dissemination of the MPV to some of the secondary and probably all tertiary target organs (Fig. 3). Viremia occurs between the 3rd and 14th day (73, 74). There is a considerable variability in the intensity and in the duration of viremia; some animals have high and others have relatively low concentrations of virus; in some monkeys viremia appears to last only several days, and in others 7 to 10 days. When clean separation of blood components (red blood cells, buffy coat, and plasma) is accomplished, MPV appears to be mainly cell-associated, particularly with the leukocytes of the buffy coat. This association can be demonstrated both by virus isolation and by the immunofluorescent method (72). Another finding worthy of note relates to the persistence of viremia beyond the time of appearance of humoral antibodies (Fig. 4). The persistence of virus in blood (and also in tissues) in the presence of antibodies suggests that MPV, because of intracellular location, may be "protected" from antibody action, or the early formed antibody molecules are incompetent for complete neutralization of these early virions.

### Histopathology

Histopathological aspects of monkeypox have been reported for both naturally occurring and experimental infections (31, 67, 71, 75). Experimentally, the inoculation of MPV intramuscularly is followed within 24 to 48 h by an intense local inflammatory response (71)

characterized by pronounced focal cell destruction, particularly of interstitial connective tissue components. Excepting this site, further cellular injury from MPV is usually not found during the preeruptive period. Foci of cell necrosis may develop in the spleen as early as the 7th day; thereafter, such foci are noted with increasing frequency in tonsils, lymph nodes, testes, ovaries, kidneys (71, 75), and in liver and lungs (31). Lesions encountered in these organs are characterized by inflammation, cellular proliferation, degeneration, and focal necrosis. Lesions involving the skin and mucous membranes are characterized by epithelial degeneration, reticulation, endothelial proliferation, inflammatory cell infiltration, necrosis, and granulation. Cytoplasmic inclusion bodies can be found; they may or may not be numerous (63, 71); intranuclear inclusions have been observed also (63). The lesions accompanying infection in monkeys from MPV are entirely like those described in human beings from infection with variola virus (25, 36, 65, 71).

### Antibody Responses

HI antibodies may be detectable as early as the 8th day after infection, almost coinciding with and occasionally preceding the earliest appearance of the skin eruption. HI antibodies reach their peak during the 3rd week, approaching titers of approximately 1:640 to 1:1,280 (Fig. 5). HI antibodies decline between the 3rd and 6th week and plateau between the 2nd and 3rd month. At 6 months, titer values range between 1:20 to 1:30. Serum-neutralizing (SN) antibodies appear concomitantly with or closely follow the development of HI antibody. SN antibody may reach peak titers by the 3rd week, or even later; the peak values are similar to HI titers and remain constant for longer than 3 months (our longest period of observation). CF antibodies appear approximately 1 week after HI antibody and decline slowly over a period of 90 to 230 days (40, 73).

Monkeys inoculated with small doses of MPV ( $\leq 600$  TCID<sub>50</sub>) showed development of lower titers of HI antibody, and also earlier peak and initial decline, than animals given large doses ( $\leq 60,000$  TCID<sub>50</sub>). This phenomenon remains unexplained, but may be due to a modulation of intracellular virus replication and switch-off of antibody formation when enough is formed to prevent further replication of infectious virus (73).

### Cross Immunity

As indicated earlier, MPV shares common antigens with vaccinia and variola viruses,

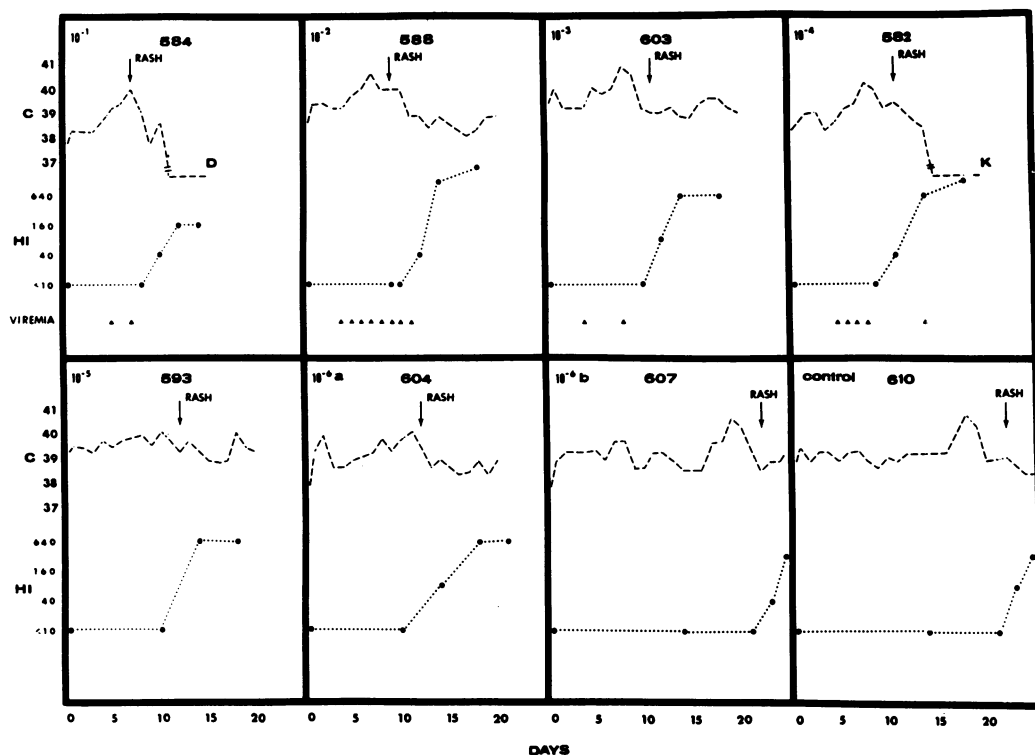


FIG. 4. Titration data on monkeypox: some clinical and laboratory findings (16, 73). *Cynomolgus* monkeys were infected with various dilutions ( $10^{-1}$  to  $10^{-6}$ ) of MPV. Eight monkeys were selected for the purpose of comparison of their clinical responses (temperature and onset of skin rash) and laboratory findings (HI antibodies and viremia). See test for discussion. Monkey 584 and 582 developed hypothermia with a body temperature of less than 35°C. D, died; K, killed. Note the delayed incubation in monkey 610. The monkey was a sentinel control and acquired monkeypox while exposed to experimentally infected monkeys housed in adjacent cages. The same may apply to monkey 607 inoculated with  $10^{-6}$  dilution of virus, a value very close to  $\leq 1$  ID<sub>50</sub>.

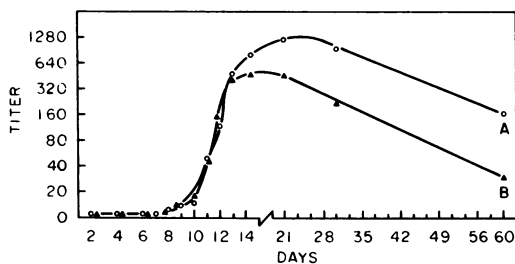


FIG. 5. Rise and fall of HI antibodies (73). Curve A (open circles) was derived from the geometric mean values obtained on six monkeys inoculated with  $10^{4.8}$  and  $10^{3.8}$  ID<sub>50</sub> of MPV; curve B (solid triangles) was derived similarly from values on seven monkeys inoculated with  $10^{3.8}$  and  $10^{1.8}$  ID<sub>50</sub>. Reproduced by permission (Arch. Gesamte Virusforsch. 27:166-178, 1969).

hence there ought to be cross-resistance on challenge. McConnell et al. (50, 52) have demonstrated that monkeypox is preventable in

rhesus monkeys and chimpanzees by intradermal inoculation of vaccinia virus. Similar cross protection for cynomolgus monkeys has also been observed by Gispén et al. (31). In our laboratory, monkeys recovering from monkeypox also resisted intradermal challenge with vaccinia virus (74, 75). In contrast, monkeys recovered from infection from monkeypox or vaccinia virus, or both, were not immune to challenge with Yaba tumor virus (74).

## SPECIAL ASPECTS

### Antiviral Compound, Methisazone

Isatin- $\beta$ -thiosemicarbazone has been used extensively to study the mechanism of action of its antiviral activity among poxviruses (44). Methisazone (1-methyl-isatin-3-thiosemicarbazone or marboran), one of the more effective derivatives, has been used in prophylactic control of smallpox (7, 8).

Methisazone has been used by us to study the cellular expression of MPV (17). In vitro studies revealed that methisazone suppressed plaque development in CV-1 cells infected with MPV. However, in vivo experiments with methisazone showed only a slight prolongation of survival time among MPV-infected chicken embryos. A temporary prolongation of survival time was also observed among treated mice inoculated intraperitoneally with MPV. No protection was afforded in mice inoculated intracerebrally. Methisazone failed to prevent the development of monkeypox in MPV-infected monkeys; although the illness seemed to be mild in intensity, the pathogenetic and immunologic features of infection were indistinguishable from those of infected control monkeys. Methisazone was used also during an outbreak of monkeypox in the Rotterdam Zoo and apparently failed to curtail the spread of the disease (60).

### Infection in Immunosuppressed Hosts

Monkeypox provides an ideal model for studies of infection and immunity. During the outbreak of monkeypox at the Walter Reed Army Institute of Research in 1962, pre-exposure irradiated monkeys died while non-irradiated animals survived the disease (49). Studies on the effects of ALS on monkeypox revealed that ALS adversely enhanced morbidity and mortality of infected monkeys (72). Azathioprine used either singly or in combination with ALS intensified the clinical and pathological responses of cynomolgus monkeys to MPV (Wenner et al., unpublished data). Among 14 inoculated monkeys (5 control animals) fever occurred in all at similar intervals ( $8 \pm 1.5$  days), as did the appearance of pocks ( $9 \pm 0.8$  days). The major differences relate to the late course of infection, where impaired healing, cutaneous gangrene of the inoculated limb, and risks of dying during a shocklike syndrome were notably greater in monkeys given either ALS or azathioprine than in the untreated controls. The significant pathological features of the dermal lesions in all treated monkeys related to modulation of the inflammatory response and delay in tissue reparative processes. Adrenal hemorrhage occurred in four treated monkeys and in one control animal. Viremia developed and persisted until death resulted in ALS-treated, but not in azathioprine-treated, monkeys. Despite this sustained viremia, concentrations of MPV in tissues corresponded with those obtained in untreated animals; moreover, the histological studies sug-

gested that cellular injury was not a major factor contributing to death. ALS temporarily delayed development of HI and CF antibodies; similar delays were not observed in azathioprine-treated animals. These data suggest that the principal area of defense vulnerability relates to suppression of cellular immunity and delayed repair, rather than humoral antibody synthetic events.

### EPIZOOTIOLOGY

Recently, attention has been paid to the possible existence of a reservoir of smallpox in nonhuman primates (5, 6, 58). Serological surveys have been made to determine the extent of specific antibodies to poxviruses in different monkey populations and their various habitats (5, 45, 58).

Several serological tests have been used providing evidence of poxvirus infection. The CF test is not entirely optimal because of frequent anticomplementary activity of monkey sera (5). The HI test is reasonably reliable, but temporal persistence of HI antibody is still largely unknown. Such antibodies may not persist longer than 1 year. SN antibody persists for a longer period ( $\leq 1$  year), but this rather tedious test is not practical for large-scale epidemiological studies.

We have presented data suggesting that subclinical poxvirus infection may be a common event in captive monkeys. McConnell et al. (49) apparently described another such occurrence. After the appearance of monkeypox in two animals, he subsequently found 25 out of 27 (93%) cynomolgus, 52 out of 67 (78%) rhesus, and 6 out of 14 (43%) African green monkeys quartered in the same compound to have HI antibodies. On the other hand, only 5 out of 45 (11%) cynomolgus monkeys housed in a separate compound had such antibody. Within this separate compound monkeypox had not been recognized.

At this time (5, 45; see Addendum) more than 2,000 sera from 20 different species of monkeys originating in Africa, India, Pakistan, Philippines, Japan, and South America have been tested for MPV antibodies, primarily using the HI and for some the SN test. The results of positive HI tests are difficult to interpret, however; only a few unequivocally positive results were obtained. Two out of 250 sera (mostly from cynomolgus, rhesus, and fuscata monkeys) showed weakly positive SN tests; both sera were obtained from cynomolgus monkeys. Additional serological surveys have been conducted by Noble (58) and Kalter and

Heberling (45). The results are summarized in Table 3. Since the source of animals and the time held in captivity are often unknown, the data are difficult to interpret also. Nevertheless, there appears to be a great deal of variation in positivity among various species of Old and New World monkeys. In the series of Kalter and Heberling, approximately 3.5% (46 out of 1,281) of the monkeys are positive for HI antibody to vaccinia virus, and 12.2% (149 out of 1,219) were positive to MPV. Noble found a comparable result: approximately 5% (26 out of 535) were positive for HI antibody to vaccinia virus. Among the monkeys we have tested (98 young cynomolgus and 13 rhesus monkeys originating from India or the Philippines), none had HI antibody to MPV upon arrival in our laboratory (17, 71-75).

As noted earlier in this review, monkeypox and vaccinia viruses share major common antigens thereby making it virtually impossible to distinguish them in conventional serologic tests. The data summarized in Table 3 reveal in general that a larger proportion of sera

procured from human beings and nonhuman primates was reactive with monkeypox than vaccinia HA. There were two exceptions involving rhesus and vervet monkeys. Assuming that the HI antibodies were engendered as a result of previous infection from a poxvirus, then the reactions in human beings presumably apply to vaccinia-variola, and those in nonhuman primates to monkeypox. While the data may be ascribed to a greater sensitivity of MPV HA, the possibility also exists that certain sera representing infections acquired more recently than others may have differing avidities that are hidden in a screening test wherein full serum dilution end points were not determined. Nevertheless, these data point up the variable but appreciable risks of infection by a poxvirus among primates; presumably this virus is MPV. Neither of the rates of HI antibodies determined for human beings or species of nonhuman primates can be considered absolute in view of the known decline of HI antibodies within several months post-infection.

TABLE 3. *Antibody to vaccinia and monkeypox viruses in human and nonhuman primates<sup>a</sup>*

Species	Screening of sera for HI antibody to			
	Vaccinia virus		Monkeypox virus	
	No. positive/no. tested	Positive (%)	No. positive/no. tested	Positive (%)
Human <sup>b</sup>	13/109	11.9	31/115	26.9
Old World monkeys				
Gorilla .....	0/39	0.0	0/38	0.0
Chimpanzee .....	4/267	1.5	34/261	13.0
Orangutan .....	0/89	0.0	1/74	1.4
Gibbon .....	0/12	0.0	0/8	0.0
Baboon .....	0/145 (3/74)	0.0 (4.0)	17/147	11.6
Rhesus .....	12/210	5.7	11/160	6.9
Cynomolgus .....	1/77 (0/64)	1.3 (0)	4/80	5.0
Japanese macaques .....	0/44	0.0	3/44	6.9
Vervet .....	4/77 (2/21)	5.2 (9.5)	3/83	3.6
Patás .....	0/55 (20/317)	0.0 (6.3)	2/52	3.5
Talapoin .....	0/21	0.0	4/22	18.2
New World monkeys				
Marmosets .....	2/43	4.7	6/43	13.9
Squirrel .....	20/63 (0/5)	31.7 (0)	37/63	58.7
Capuchin .....	0/14 (1/4)	0.0 (25.0)	1/2	50.0
Woolly .....	0/1 (0/11)	0.0 (0)	4/12	33.3
Owl .....	0/8	0.0	0/8	0.0
Spider .....	3/7 (0/11)	42.9 (0)	7/7	100.0

<sup>a</sup> These data are compiled from Kalter and Herling (45); data in parentheses are obtained from Noble (58). These represent results of sera obtained from various parts of the world, some having been tested in varied laboratories. The majority of the sera were tested for the presence or absence of antibody to poxviruses by using both vaccinia and monkeypox antigens.

<sup>b</sup> Sources of human sera are from Kenya, Tex., U. S. Army recruits, etc. The vaccination status of these men is unknown.

## CLINICAL AND EPIDEMIOLOGICAL IMPLICATIONS

### Monkeys as a Reservoir for Smallpox?

The absence of an animal reservoir of variola virus is essential for the success of world-wide smallpox eradication. Smallpox appears to be highly host-specific; besides human beings only a few primate species are known to be susceptible to infection (5, 14, 36, 58).

Arita and Henderson (6) recently reviewed the problems of smallpox and monkeypox in nonhuman primates. They, along with Noble (58), conclude that a natural reservoir for smallpox in nonhuman primates is unlikely. Supportive evidences used in arriving at this conclusion are: (i) epidemiological surveillance indicates that outbreaks of smallpox in monkeys are rare phenomena; only 7 such episodes are reported, and no outbreak has been recognized since 1936 (6); (ii) human smallpox has virtually disappeared from several geographical areas containing large monkey populations, e.g., Panama and the Philippines; (iii) serological surveys of monkeys for evidence of poxvirus infection reveal that usually only a fraction of the animals exhibit HI antibody to poxviruses (5, 36, 45, 58); and (iv) although cynomolgus monkeys are susceptible to smallpox, the disease is not readily transmissible in serial passages (58). However, the susceptibility of certain species of monkeys to smallpox (35, 36, 58, 76) and the recent recognition of the closely related virus of monkeypox (70) warrant further studies and continued field observations (5, 6) particularly with respect to (i) circumscribed, exogenous (nonhuman) reservoirs of variola virus and (ii) the epizootiology of monkeypox.

### Monkeypox in Man

There appeared to be virtually no risk of human infection among personnel exposed during the various outbreaks of monkeypox (see Historical Note). The initial impression was that human beings may be comparatively insusceptible to MPV (6, 65). However, most if not all such persons had been vaccinated against smallpox. Now it has become clear that natural infection with MPV may occur in human beings (3, 4). Although the primary source of infection in the small outbreaks occurring in South Africa was not identified, the information available suggests primary transmission of MPV from monkeys to human beings. Since the clinical features of these human infections resembled smallpox, difficul-

ties in differential diagnosis can be anticipated and discrete outbreaks of monkeypox may remain unrecognized. At this time there is no solid evidence for the transmission of MPV from person to person; however, opportunity for critical study of clusters of human infection have been too few to establish the epidemiological pattern (see Addendum). Having at hand information on the relative ease of cross-infection between captive monkeys, and recognizing as yet no biological reason why similar events should not prevail for susceptible (unvaccinated) members of the human population, we look forward to the further development of critical studies relating MPV to infections among children and adults residing in enzootic areas.

Moreover, MPV has been recovered from the tissue cultures derived from the kidneys of apparently healthy cynomolgus monkeys (31), and seroconversion has been defined among monkeys with inapparent infections; therefore, "silent" infections from MPV in nature may be more common (5) than is generally appreciated! The demonstrable presence of MPV in captive monkeys should alert those concerned with usage of tissue cultures in the field of biological research to the potentiality of MPV as a risk to human beings. Hence, it appears advisable to protect persons against infection from such poxviruses by vaccination, especially those who handle monkeys or work with biological materials involving tissue cultures of primate species.

## CONCLUSIONS

MPV was isolated in 1958 during outbreaks of a pox disease in laboratory colonies of cynomolgus monkeys in Copenhagen. Since then, several outbreaks have occurred in different species of non-human primates housed in laboratories in various parts of the world. Naturally occurring infections among monkeys in their native habitat is unknown; however, the appearance of infection by MPV in children residing in West Africa suggests that wild monkeys (or related species) are the likely harborers of MPV.

Studies on the biological properties of MPV indicate that it is closely related to the *vacinia-variola* subgroup of poxviruses. MPV produces pock lesions on the CAM of developing chicken embryos, encephalitis in mice, and characteristic dermal lesions and keratitis in rabbits. Morphologically, the virus is 200 by 250 nm in size and has a rectangular shape. Many mammalian cells in culture support the

growth of MPV. Cytopathic effects and plaque formation can be easily produced in cultured cells.

Clinically, monkeypox as found in monkeys and in human beings cannot be differentiated from variola. Serological data support the occurrence of subclinical infection in monkeys; the ratio of subclinical-clinical infection is unknown. In its pathogenesis monkeypox follows patterns described for variola, ectromelia, and rabbitpox. Histopathological features of infection are similar to those found in human infections from variola virus, to wit: inflammation, cellular proliferation, degeneration, and focal cellular necrosis in various organ systems including the dermis. Specific antibodies are engendered in infected hosts and these may be demonstrated by using HI, CF, and SN tests. MPV shares common antigens with vaccinia and variola. Therefore, there is a strong serological cross-reactivity and clinical cross-immunity between them.

Serological surveys to determine the frequency of specific antibody to poxviruses in different monkey populations show wide ranges between species; on the average, less than 12% of monkeys originating from different parts of the world contain HI antibody. Whether acquisition of infection was in their native habitat, or followed captivity, remains unknown. Epidemiological surveillances suggest that a natural reservoir of smallpox in nonhuman primates is unlikely; however, further observations are needed, particularly with respect to monkey populations with high infection rates based on existent HI antibodies.

MPV is pathogenic to man; this feature undoubtedly has clinical and epidemiological significance. Protection of human beings against monkeypox by vaccination with vaccinia virus is mandatory among those who handle monkeys or tissue cultures of primate species.

Unquivocal answers cannot be given to questions asked in the introductory section. If monkeypox is variola, the contagiousness is exceptional, for variola virus is not easily acquired by sentinel companions quartered among infected monkeys. If infection relates to vaccinia virus, it is unique also, for to our knowledge generalized vaccinia has never been reported in healthy primates (immunologically and dermatologically intact). If it is a hybrid virus of recent or remote origin derived by reactivation in vivo of variola and another poxvirus, it is yet to be described. Finally, if it is uniquely a primary poxvirus (MPV) of monkeys, as it well might be, many of its principal

properties have been defined.

### ADDENDUM

After this review was written, a series of papers appeared in the Bulletin of the World Health Organization (vol. 46, p. 567-639, 1972). None of these reports significantly alters the data presented herein, but they do amplify several points of discussion. At least two more human infections have been recognized (Ladnyj et al., p. 593-597; Bourke and Dumbell, p. 621-653). The clinical characteristics of MPV infection in humans is described by Foster et al., p. 569-570. The epidemiological data point out the low, and possibly negligible, risk of human-to-human transmission; some susceptibles at risk were successfully vaccinated, suggesting little if any immunity to vaccinia virus. Studies on properties of MPV suggest that (i) the virus is a specific homogeneous poxvirus (Rondle and Sayeed, p. 577-583) or (ii) heterogeneous, since mutants closely related to variola have been selected from strains isolated from healthy monkeys. Thus the most recent published data leave unanswered most of those questions posed in the introductory section of this review.

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### LITERATURE CITED

1. Ambrus, J. L., E. T. Feltz, J. T. Grace, and G. Owens. 1963. A virus induced tumor in primates. *Nat. Cancer Inst. Monogr.* 10:447-458.
2. Anonymous. 1968. The smallpox eradication programme. *WHO Chronicle* 22:354-362.
3. Anonymous. 1971. Human infection with monkeypox virus. *Morbidity and Mortality Weekly Report*, vol. 20, no. 8.
4. Anonymous. 1971. A rare smallpox-like disease. *WHO Chronicle* 25:370-372.
5. Arita, I., F. Fenner, R. Gispén (Chairman), S. B. Gurrich, S. S. Kalter, S. S. Marennikova, G. Meiklejohn, J. Noble, Jr., G. M. Sheluchina, V. D. Soloviev, and I. Tayaga. 1968. Report of informal discussion on monkeypox virus studies. Draft to report to WHO.
6. Arita, I., and D. A. Henderson. 1969. Smallpox and monkeypox in non-human primates. *Bull. WHO* 39:277-283.
7. Bauer, D., L. St. Vincent, C. H. Kempe, and A. W. Downie. 1963. Prophylactic treatment of smallpox contacts with N-methylisatin-beta-thiosemicarbazone (compound 33T57 marboran). *Lancet* 2:494-496.
8. Bauer, D. J., L. St. Vincent, and H. Kempe.

1969. Prophylaxis of smallpox with methisazone. *Amer. J. Epidemiol.* **90**:130-145.
9. Baxby, D., and B. J. Hill. 1971. Characteristics of a new poxvirus isolated from indian bufaloes. *Arch. Gesamte Virusforsch.* **35**:70-79.
10. Bearcroft, W. G. C., and M. F. Jamieson. 1958. An outbreak of subcutaneous tumours in rhesus monkeys. *Nature (London)* **182**:195-196.
11. Bedson, H. S., and K. R. Dumbell. 1961. The effect of temperature on the growth of pox viruses in chick embryo. *J. Hyg. (Camb.)* **59**:457-469.
12. Bedson, H. S., and J. J. Duckworth. 1963. Rabbitpox: an experimental study of the pathways of infection in rabbits. *J. Pathol. Bacteriol.* **85**:1-20.
13. Bleyer, J. C. 1922. Über Auftreten von Variola unter Affen der Genera *Myctes* und *Cebus* bei Vordringen einer Pockenepidemie im Urwaldgebiete an den Nebenflüssen des Alto Uruguay in Sudbrasilien. *Münch. Med. Wochschr.* **69**:1009-1010.
14. Brinckerhoff, W. R., and E. E. Tyzzer. 1905-06. Studies on experimental variola and vaccinia in *Quadrumanus*. *J. Med. Res.* **9**:223-359.
15. Casey, H. W., J. M. Woodruff, and W. I. Butcher. 1967. Electron microscopy of a benign epidermal pox disease of rhesus monkeys. *Amer. J. Pathol.* **51**:431-446.
16. Cho, C. T. Biological activity of monkeypox virus: studies on growth characteristics in cell culture, pathogenesis in monkeys and modification of infection by methisazone. Dissertation Abstracts International, vol. 31, no. 11, p. 6773-B. University Microfilms, Ann Arbor, Mich.
17. Cho, C. T., C. R. Bolano, P. S. Kamitsuka, and H. A. Wenner. 1970. Methisazone and monkeypox virus: studies in cell cultures, chick embryos, mice and monkeys. *Amer. J. Epidemiol.* **92**:137-144.
18. Cho, C. T., C. Liu, and H. A. Wenner. 1972. Monkeypox virus: responses of infection in chick embryos. *Proc. Soc. Exp. Biol. Med.* **139**:1206-1212.
19. Cho, C. T., T. Locke, and H. A. Wenner. 1970. Viremia and virus measurements of rabbitpox in CV-1 cells. *Appl. Microbiol.* **19**:791-794.
20. Cho, C. T., and H. A. Wenner. 1972. In vitro growth characteristics of monkeypox virus. *Proc. Soc. Exp. Biol. Med.* **139**:916-920.
21. Chu, C. M. 1948. Studies on vaccinia hemagglutinin. I. Some physicochemical properties. *J. Hyg.* **46**:42-48.
22. Crandell, R. A., H. W. Casey, and W. B. Brumlow. 1969. Studies of a newly recognized poxvirus of monkeys. *J. Infect. Dis.* **119**:80-88.
23. Dales, S. 1963. The uptake and development of vaccinia virus in strain L cells followed with labeled viral deoxyribonucleic acid. *J. Cell Biol.* **18**:51-72.
24. Daniel, M. D., A. Karpas, L. V. Melendez, N. W. King, and R. D. Hunt. 1967. Isolation of herpes-T virus from a spontaneous disease in squirrel monkeys (*Saimiri sciureus*). *Arch. Gesamte Virusforsch.* **22**:324-331.
25. Downie, A. W. 1965. Poxvirus group, p. 932-964. In F. L. Horsfall and J. Tamm (ed.), *Viral and rickettsial infections of man*. 4th ed., J. B. Lippincott Co. Philadelphia.
26. Downie, A. W., C. H. Taylor-Robinson, A. E. Caunt, G. S. Nelson, P. E. C. Manson-Bahr, and T. C. H. Matthews. 1971. Tanapox: a new disease caused by a pox virus. *Brit. Med. J.* **1**:363-368.
27. Douglas, J. D., K. N. Tanner, J. R. Prine, D. C. Van Riper, and S. K. Derwelis. 1967. *Molluscum contagiosum* in chimpanzees. *J. Amer. Vet. Med. Ass.* **151**:901-904.
28. Fenner, F. 1949. Mouse-pox (infectious ectromelia of mice). A review. *J. Immunol.* **63**:341-373.
29. Fenner, F. 1958. The biological characteristics of several strains of vaccinia, cowpox and rabbitpox viruses. *Virology* **5**:502-529.
30. Furness, G., and J. S. Younger. 1959. One-step growth curves of vaccinia virus in cultures of monkey kidney cells. *Virology* **9**:386-395.
31. Gispén, R., J. D. Verlinde, and P. Zwart. 1967. Histopathological and virological studies on monkeypox. *Arch. Virusforsch.* **21**:205-216.
32. Grace, J. T., and E. A. Mirand. 1963. Human susceptibility to a simian tumor virus. *Ann. N.Y. Acad. Sci.* **108**:1123-1128.
33. Grace, J. T., E. A. Mirand, S. J. Millan, and R. S. Metzger. 1962. Experimental studies on human tumors. *Fed. Proc.* **21**:32-36.
34. Hahon, N. 1958. Cytopathogenicity and propagation of variola virus in tissue culture. *J. Immunol.* **81**:426-432.
35. Hahon, N., and B. J. Wilson. 1960. Pathogenesis of variola in *Macaca irus* monkeys. *Amer. J. Hyg.* **71**:69-80.
36. Hahon, N. 1961. Smallpox and related poxvirus infections in the simian host. *Bacteriol. Rev.* **25**:459-481.
37. Hahon, N., and M. H. McGavran. 1961. Airborne infectivity of the variola-vaccinia group of poxviruses for the cynomolgus monkey. *Macaca irus*. *J. Infect. Dis.* **109**:294-298.
38. Hall, A. S., and W. P. McNulty. 1967. A contagious pox disease in monkeys. *J. Amer. Vet. Med. Ass.* **151**:833-838.
39. Heberling, R. L., J. Eichberg, and S. S. Kalter. 1972. The course of monkeypox virus infection in conventional and gnotobiotic baboons (*Papio cynocephalus*). *Abstr. Annu. Meeting Amer. Soc. Microbiol.*, p. 233.
40. Heberling, R. L., and S. S. Kalter. 1971. Induction, course and transmissibility of monkeypox in the baboon (*Papio cynocephalus*). *J. Infect. Dis.* **124**:33-38.
41. Henderson, D. A. 1971. Official Records of the World Health Organization, no. 194, p. 408.
42. Horgan, E. S., and M. A. Haseeb. 1939. Cross immunity experiments in monkeys between variola, alastrim and vaccinia. *J. Hyg.* **39**:615-637.
43. Hull, R. N. 1969. The significance of simian viruses to the monkey colony and the labora-



- tory investigator. *Ann. N.Y. Acad. Sci.* **162**:472-482.
44. Joklik, W. K. 1966. The poxviruses. *Bacteriol. Rev.* **30**:33-66.
  45. Kalter, S. S., and R. L. Heberling. 1971. Comparative virology of primates. *Bacteriol. Rev.* **35**:310-364.
  46. Loh, P. C., and J. L. Riggs. 1961. Demonstration of the sequential development of vaccinia antigens and virus in infected cells: observations with cytochemical and differential fluorescent procedures. *J. Exp. Med.* **114**:149-160.
  47. Magrath, G. B., and W. R. Brinckerhoff. 1904. On experimental variola in the monkey. *J. Med. Res.* **6**:230-246.
  48. Marennikova, S. S., E. B. Gurvich, and E. M. Shelukhina. 1971. Comparison of the properties of five pox virus stains isolated from monkeys. *Arch. Gesamte. Virusforsch.* **33**:201-210.
  49. McConnell, S. J., Y. F. Herman, D. E. Mattson, and L. Erickson. 1962. Monkeypox disease in irradiated cynomolgus monkeys. *Nature (London)* **195**:1128-1129.
  50. McConnell, S., Y. F. Herman, D. E. Mattson, D. L. Huxsoll, C. M. Lang, and R. H. Yager. 1964. Protection of rhesus monkeys against monkeypox by vaccinia virus immunization. *Amer. J. Vet. Res.* **25**:192-195.
  51. McConnell, S. J., R. O. Spertzel, D. L. Huxsoll, L. H. Elliott, and R. H. Yager. 1964. Plaque morphology of monkeypox virus as an aid to strain identification. *J. Bacteriol.* **87**:238-239.
  52. McConnell, S., R. L. Hickman, W. L. Wooding, and D. L. Huxsoll. 1968. Monkeypox: experimental infection in chimpanzee (*Pan satyrus*) and immunization with vaccinia virus. *Amer. J. Vet. Res.* **29**:1675-1680.
  53. McNulty, W. P., W. C. Lobitz, F. Hu, C. A. Maruffo, and A. S. Hall. 1968. A pox disease in monkeys transmitted to man. *Arch. Dermatol.* **97**:286-293.
  54. Melnick, J. L. 1971. Classification and nomenclature of animal viruses. *Progr. Med. Virol.* **13**:462-484.
  55. Middelkamp, J. N., and H. A. Wenner. 1969. Electron microscopy of monkeypox virus in epidermal lesions. *Bacteriol. Proc.*, p. 190.
  56. Mika, L. A., and J. B. Pirsch. 1960. Differentiation of variola from other members of the poxvirus group by the plaque technique. *J. Bacteriol.* **80**:861-863.
  57. Nicholas, A. H., and W. P. McNulty. 1968. In vitro characteristics of a poxvirus isolated from rhesus monkeys. *Nature (London)* **217**:745-746.
  58. Noble, J., Jr. 1970. A study of new and old world monkeys to determine the likelihood of a simian reservoir of smallpox. *Bull. WHO* **42**:509-514.
  59. Noble, J., and J. A. Rich. 1969. Transmission of smallpox by contact and by aerosol routes in *Macaca irus*. *Bull. WHO* **40**:279-286.
  60. Peters, J. C. 1966. Eine "Monkey-Pox" enzootie im affenhaus des Tiergartens "Blijdorp." *Kleint.-Prax.* **11**:65-71.
  61. Peters, J. C. 1966. A monkeypox-enzooty in the Blijdorp Zoo. *Tijdschr. Diergeneesk* **91**:387-391.
  62. Pirsch, J. B., L. A. Mika, and E. H. Purlson. 1963. Growth characteristics of variola virus in tissue culture. *J. Infect. Dis.* **113**:170-187.
  63. Prier, J. E., and R. M. Sauer. 1960. A pox disease of monkeys. *Ann. N.Y. Acad. Sci.* **85**:951-959.
  64. Prier, J. E., R. M. Sauer, R. G. Malsberger, and J. M. Sillaman. 1960. Studies on a pox disease of monkeys. II. Isolation of the etiologic agent. *Amer. J. Vet. Res.* **21**:381-384.
  65. Rhodes, A. J., and C. E. van Rooyen. 1968. The poxvirus group: general properties, p. 299-311. In *Textbook of virology*. The Williams & Wilkins Co., Baltimore.
  66. Rouhandeh, H., R. Engler, M. T. A. Fouad, and L. L. Sells. 1967. Properties of monkeypox virus. *Arch. Virusforsch.* **20**:363-373.
  67. Sauer, R. M., J. E. Prier, R. S. Buchanan, A. A. Creamer, and H. C. Fegley. 1960. Studies on a pox disease of monkeys. I. Pathology. *Amer. J. Vet. Res.* **21**:377-380.
  68. Sheek, K. O., and W. E. Magee. 1961. An autoradiographic study on the intracellular development of vaccinia virus. *Virology* **15**:146-163.
  69. Smith, K. O., and D. G. Sharp. 1960. Interaction of virus with cells in tissue cultures. I. Adsorption on and growth of vaccinia virus in L cells. *Virology* **11**:519-532.
  70. von Magnus, P., E. K. Anderson, K. B. Peterson, and A. Birch-Anderson. 1959. A pox-like disease in cynomolgus monkeys. *Acta Pathol. Microbiol. Scand.* **46**:156-176.
  71. Wenner, H. A., C. R. Bolano, C. T. Cho, and P. S. Kamitsuka. 1969. Studies on the pathogenesis of monkeypox. III. Histopathological lesions and sites of immunofluorescence. *Arch. Gesamte Virusforsch.* **27**:179-197.
  72. Wenner, H. A., C. Bolano, C. T. Cho, and P. S. Kamitsuka. 1969. Monkeypox. IV. Modification of disease pattern by antilymphocytic sera. *J. Infect. Dis.* **120**:318-331.
  73. Wenner, H. A., C. T. Cho, C. R. Bolano, and P. S. Kamitsuka. 1969. Studies on the pathogenesis of monkeypox. II. Dose-response and virus dispersion. *Arch. Gesamte Virusforsch.* **27**:166-178.
  74. Wenner, H. A., P. S. Kamitsuka, F. D. Macasaet, and P. Kidd. 1967. Pathogenesis of monkeypox, p. 40-44. *Antimicrobiol. Ag. Chemother.* 1966. American Society for Microbiology, Ann Arbor, Mich.
  75. Wenner, H. A., F. D. Macasaet, P. S. Kamitsuka, and P. Kidd. 1968. Monkeypox. I. Clinical, virologic and immunologic studies. *Amer. J. Epidemiol.* **87**:551-566.
  76. Westwood, J. C. N., E. A. Boulter, E. T. W. Bowen, and H. B. Maber. 1966. Experimental respiratory infection with poxviruses. I. Clinical, virological and epidemiological studies. *Brit. J. Exp. Pathol.* **47**:453-465.

77. Woodroffe, G. J., and F. Fenner. 1962. Serological relationships within the pox virus group: an antigen common to all members of the group. *Virology* **16**:334-341.
78. Woodson, B. 1968. Recent progress in poxvirus research. *Bacteriol. Rev.* **32**:127-137.
79. Yau, T., and H. Rouhandeh. 1970. Uncoating of monkeypox virus (MPV) in continuous African green monkey kidney cell line, CV-1. *Bacteriol. Proc.*, p. 204.